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Chemical Reactivity-Absorption, Retention, Metabolism, and Elimination of Hexachlorocyclopentadiene

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Hexachlorocyclopentadiene is eliminated from the body efficiently by urinary excretion. Although direct evidence is not available, it appears that expiration my be another important route of elimination. Of the administered dose 9% is excreted in the bile in 1 hr, approximately equal to the amount of fecal excretion in 7 days, suggesting enterohepatic circulation. After a single dose, HCPD decays from the blood biexponentially with a terminal phase half-life of 60 min. Of the tissues analyzed kidney, followed by liver, concentrate HCPD 1 hr or 7 days after exposure to any significant extent. Subcellularly, HCPD is predominantly associated with cytosol fractions of both kidney as well as liver, observations consistent with rapid elimination of HCPD after a single exposure. Preexposure to HCPD (50 mg/kg-day for 3 days) resulted in unaltered blood decay curves and biliary excretion, but increased the concentration in the kidneys after a single subsequent challenge.

Introduction

Hexachlorocyclopentadiene (HCPD) (I) is prechlorinated, highly reactive compound, used as a building block in chemicals having biocidal and fire-retarding features. It is used in the manufacture of most commonly used cyclodiene pesticides such as chlordane, heptachlor (Table 1), heptachlor epoxide (*1*), etc. In addition, mirex, a pesticide used in the control of fire ants, and its analog kepone, are also synthesized by condensing two molecules of HCPD (*1*). Fungicidal properties of HCPD have also been reported (*2*). It is also used in the synthesis of chlorendic anhydride and acid, chemicals comprising the basis for chemical and high-heat resistant coatings and resins with flame retardant properties. Mirex, for example, possesses flame-retardant properties in addition to insecticidal activity (*3*). A number of other industrial chemicals are formed from adduct products of HCPD with aliphatic (*4*) and aromatic dienophilic compounds (*1*, *5*). Other adduct products of HCPD with polynuclear aromatic hydrocarbons such as naphthalene and arthracene form the basis for subsequent substitution of the aromatic rings to yield several compounds used in the dye industry (*5*). HCPD is also used as a vulcanizing agent for rubber and diene elastomers (1). Although the annual U. S. production and usage is not a matter of published record, a guarded estimate of 50 million pounds may be made based on the known annual rates of production of various cyclodiene insecticides (*6*).

Table 1. Comparative toxicities of HCPD and some cyclodiene insecticides.

Pesticide	Oral LD_{50}, mg/kg		Ratio of LD_{50} to maximum tolerated dose	
	Rats	Mice	Rats	Mice
Allodan	940	750	1.9	1.5
Chlordan	500	225	1.6	4.5
Thiodan	105	75	2.4	3.7
Heptachlor	82	210	3.3	4.2
Aldrin	42	18	1.4	1.8
Dieldrin	40	24	1.3	2.4
Isodrin	10	9	2.5	2.9
Endrin	9	9	1.7	3.0
HCPD	300	505	N.A.	N.A.

[a]Data of Spynu (*7*).

Chemical Reactivity

The Diels-Alder reaction is a well-known condensation reaction in which a compound containing

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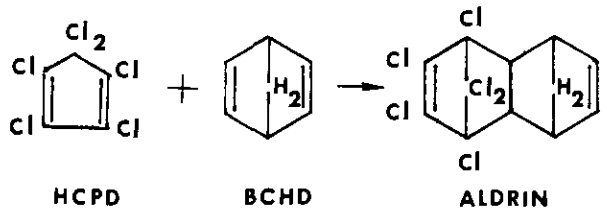
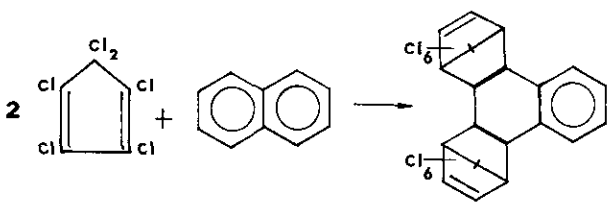
diene group forms an adduct with another double-bonded compound. HCPD shows an unexpected tendency to undergo the Diels-Alder reaction with many dienophiles at temperatures between 20° and 200°C. It condenses even with simple olefins, which normally do not react with dienes and with polynuclear aromatic hydrocarbons such as naphthalene and anthracene. Examples of such adduct products are numerous (*1*, *5*). Equimolar proportions of HCPD and bicycloheptadiene (BCHD) [Eq. (1)] react to give hexachlorotetracyclododecadiene (Aldrin) which is a parent compound for a series of cyclodiene insecticides. Another example of



(1)

Diels-Alder reaction may be seen in the dihexachloropentadiene adduct (DHA) formed from HCPD and naphthalene [Eq. (2)]. The adducts with similar polynuclear aromatics are used as starting materials in the synthesis of variously (e.g., nitration, sulfonation, chlorination and other mixed reactions) substituted intermediates used in the dye industry and other industrial applications.



(2)

Absorption, Metabolism and Excretion

HCPD-U- ^{14}C (5 μmole, 1 μCi per animal) was administered to four male Sprague-Dawley rats (225-250 g) by oral intubation as a solution in 0.2 ml corn oil. The animals were maintained in metabolism cages with food and water *ad libitum*. Daily urine and fecal samples were collected. After 7 days the animals were sacrificed and major tissues and organs were removed, homogenized in distilled water and radioassayed. Urine and powdered feces samples were also radioassayed for total ^{14}C.

Figures 1 and 2 illustrate the pattern of urinary and fecal excretion of the ^{14}C material derived from HCPD-^{14}C. An average of approximately 33% of the administered dose was excreted in the urine after 7 days (Fig. 1). About 87% of that was eliminated during the first 24 hr after the administration of HCPD. Fecal excretion accounted for about 10% of the administered dose (Fig. 2). Nearly 60% of the 7-day fecal excretion occurred during the day 1, and after the day 3 only trace amounts of HCPD-derived ^{14}C was eliminated in the feces. Tissues retained only trace amounts of HCPD after 7 days. Kidney retained 0.5% of the dose followed by liver which contained less than 0.5% of the dose.

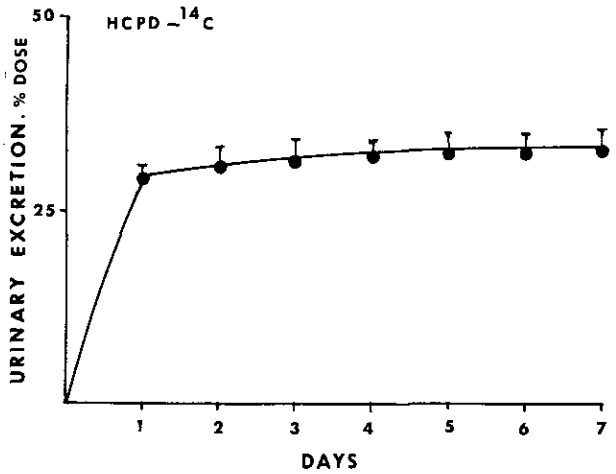


FIGURE 1. Urinary excretion of HCPD-^{14}C/derived radiolabel in male rats. Four animals were administered HCPD-^{14}C (1 μCi, 6 mg/kg) orally and housed in individual metabolism cages. Daily urine samples were analyzed for total radiolabel.

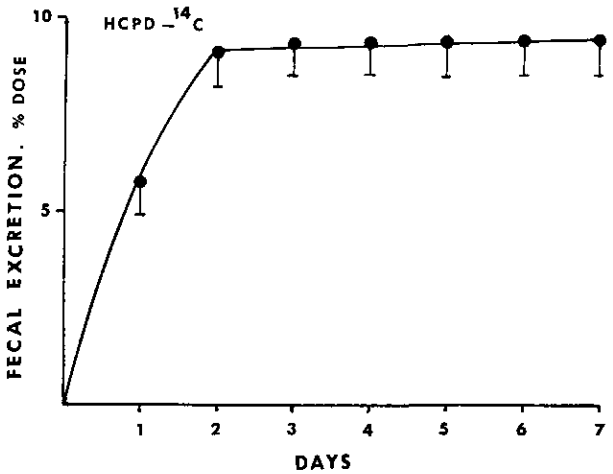


FIGURE 2. Fecal excretion of HCPD-^{14}C-derived radiolabel in male rats. Four animals were administered HCPD-^{14}C (1 μCi, 6 mg/kg) orally and housed in individual metabolism cages. Daily fecal samples were analyzed for total radiolabel.

Other tissues—fat, lung, muscle, blood, etc.—had smaller traces of the radiolabel, suggesting that perhaps better than half of the administered HCPD was eliminated by routes other than urine and feces. It is assumed that elimination by respiratory tract, followed by urinary and fecal routes, constitute major routes of elimination for HCPD.

Metabolism

Since urine contained approximately one third of the administered HCPD, the nature of the radioactivity excreted in urine was examined for possible metabolites. Urine was twice extracted with two volumes of (9:1) hexane; isopropanol mixture. Approximately 70% of the radioactivity in urine was organo-extractable leaving the remainder as water soluble material. The organic extract was concentrated and applied on silica gel G TLC plates. The plates were developed in three solvent systems: (1) cyclohexane–acetone, 80:20; (2) benzene–acetone, 9:1; (3) hexane–isopropanol, 9:1. The radioactive spots were visualized by autoradiography on medical x-ray film. These results suggest at least four metabolites of HCPD. These metabolites have not been identified and characterized. The quantitative aspects of these and water-soluble metabolites of HCPD will be the subject of future investigations.

Disposition and Biliary Excretion of HCPD-^{14}C

Male rats were anesthesized with pentobarbital sodium (45 mg/kg, IP) and femoral vein, femoral artery, and common bile duct were cannulated. Approximately 1 μCi (5 μmole) of HCPD-^{14}C was injected via femoral vein. Timed samples of blood and bile were collected for 1 hr. The radioactivity from blood decayed biexponentially (Fig. 3) with the terminal phase half-life of 1 hr. Approximately 9% of the administered dose was excreted in the bile in 1 hr (Fig. 4). The nature of the ^{14}C material in the bile has not been examined.

In other experiments, effect of pre-exposure to HCPD (50 mg/kg/day for 3 days, PO, in corn oil) on the biliary excretion of HCPD-^{14}C derived radiolabel was investigated. Biliary excretion of HCPD-^{14}C was unaltered in these experiments. Also, no discernible changes were observed in the blood concentration decay curve for HCPD-^{14}C.

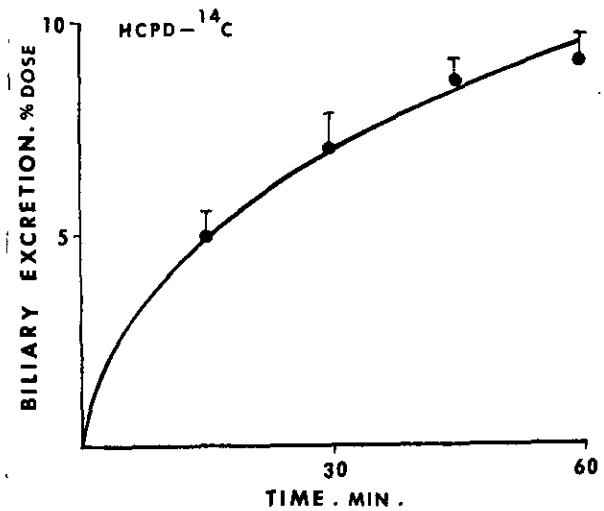


FIGURE 3. Biexponential decay of HCPD-^{14}C in the blood of male rats. Rats were cannulated under pentobarbital (45 mg/kg, IP) anesthesia. HCPD-^{14}C(1 μCi, 5 μmole) was injected through the femoral vein, and timed blood samples obtained through femoral artery were analyzed for total radiolabel. Results presented are average of three individual determinations.

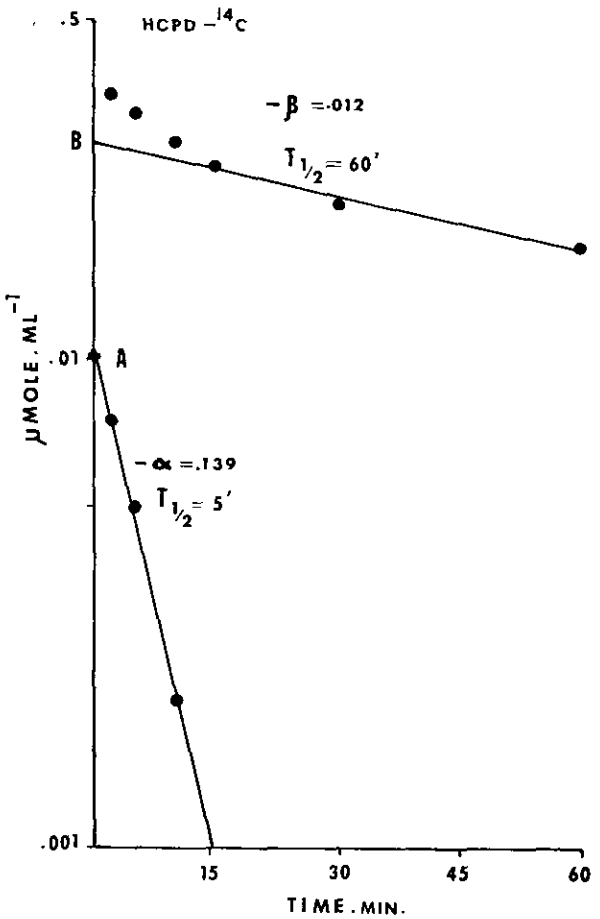


FIGURE 4. Biliary excretion of HCPD-^{14}C. Animals were cannulated under pentobarbital (45 mg/kg, IP) anesthesia. HCPD-^{14}C (1 μCi, 5 μmole) was injected via femoral vein, and timed bile samples were collected via common bile duct and analyzed for total radiolabel content. Results are means ± SEM of three individual determinations.

Subcellular Distribution of HCPD-^{14}C

At the end of the above experiments animals were sacrificed and liver and kidneys were removed. Total ^{14}C content of these organs was estimated by radioassaying samples of tissue homogenates. The distribution of radioactivity in various subcellular fractions was examined by radioassaying various centrifugation fractions of the liver and kidney homogenates. The kidney contained higher concentration of HCPD than the liver, although by virtue of the size, liver contained more HCPD equivalents (Table 2). HCPD-equivalents in various subcellular fractions were expressed as percentage of the total in the whole homogenate (Tables 3 and 4). Kidney cytosol (105,000*g* supernatant) fraction contained over 93% of the radioactivity (Table 3). These data are consistent with the rapid urinary excretion of this compound. Although to a slightly lesser extent, most of the radiolabel (68%) was present in the cytosol fraction (105,000*g* supernatant) (Table 4) of the liver. Pre-exposure to the HCPD (50 mg/kg/day for 3 days, PO) resulted in increased concentration of HCPD in the kidneys after a single challenge of HCPD-^{14}C (Table 5). Despite the larger size of the liver, kidneys retained similar amounts of HCPD-^{14}C material in terms of total quantity. The hepatic concentration of HCPD-^{14}C was unaltered by pre-exposure to HCPD. The time course of the subcellular distribution has not been examined. Also additional information may be obtained on the nature of binding, etc., by dialysis and extraction of the cytosol fractions.

HCPD appears to be eliminated from the body efficiently by urinary excretion. Although direct evidence is not available, it appears that elimination by expiration may be the major route of removal of this compound. Subcellularly HCPD is predominantly associated with liver and kidney cytosol fractions. HCPD is metabolized to at least four metabolites. Although to a small extent, HCPD is also eliminated via biliary excretion. Kinetics of absorption, via lungs and pulmonary elimination and via kidney as well as any effects of chronic exposure on these organ systems should be examined in future experiments.

Table 2. Renal and hepatic concentration of HCPD.[a]

Organ	HCPD-^{14}C after 60 min, μmole × 10^2	
	Per organ	Per g tissue
Liver	26 ± 2.0	2 ± 0.1
Kidney	14 ± 0.5	5 ± 0.3

[a]Tissue conents were determined 60 min after IV administration of 5 μmole HCPD-^{14}C. Results are means ± SEM of four individual determinations.

Table 3. Subcellular distribution of HCPD-^{14}C in kidney fractions.[a]

Fraction	Concn of kidney (μmole/g), % of ^{14}C in whole homogenate
Whole homogenate	100
900 *g*	2.1 ± 0.7
9000 *g*	3.5 ± 0.7
105,000 *g*	0.6 ± 0.2
105,000 *g* supernatant	93.8 ± 1.1

[a]At 60 min after IV administration. Results are means ± SEM of four individual determinations.

Table 4. Subcellular distribution of HCPD-^{14}C in liver fractions.[a]

Fraction	Concn of kidney (μmole/g), % of ^{14}C in whole homogenate
Whole homogenate	100
900 *g*	11.1 ± 1.9
9000 *g*	7.1 ± 2.0
105,000 *g*	14.2 ± 2.1
105,000 *g* supernatant	67.5 ± 2.2

[a]At 60 min after IV administration. Results are means ± SEM of four individual determinations.

Table 5. Effect of pre-exposure on HCPD disposition.[a]

Organ	HCPD-^{14}C after 60 min, μmole × 10^2	
	Per organ	Per g of tissue
Liver	25 ± 1	1.7 ± 0.1
Kidney	29 ± 3	8.3 ± 0.2

[a]Rats were pretreated with HCPD (50 μg/kg, PO, 3 days). Tissue contents were determined 60 min after IV administration of 5 μmole HCPD-^{14}C. Results are means ± SEM of three individual determinations.

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